Probing the Deep Biosphere:

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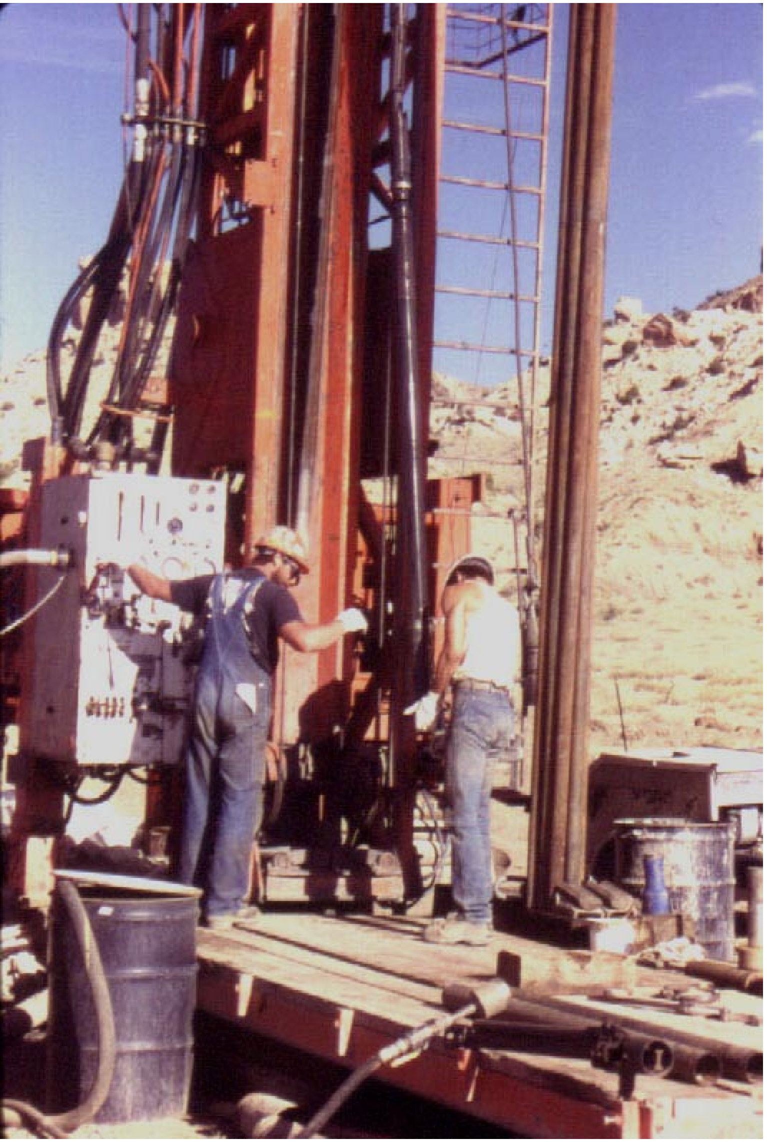
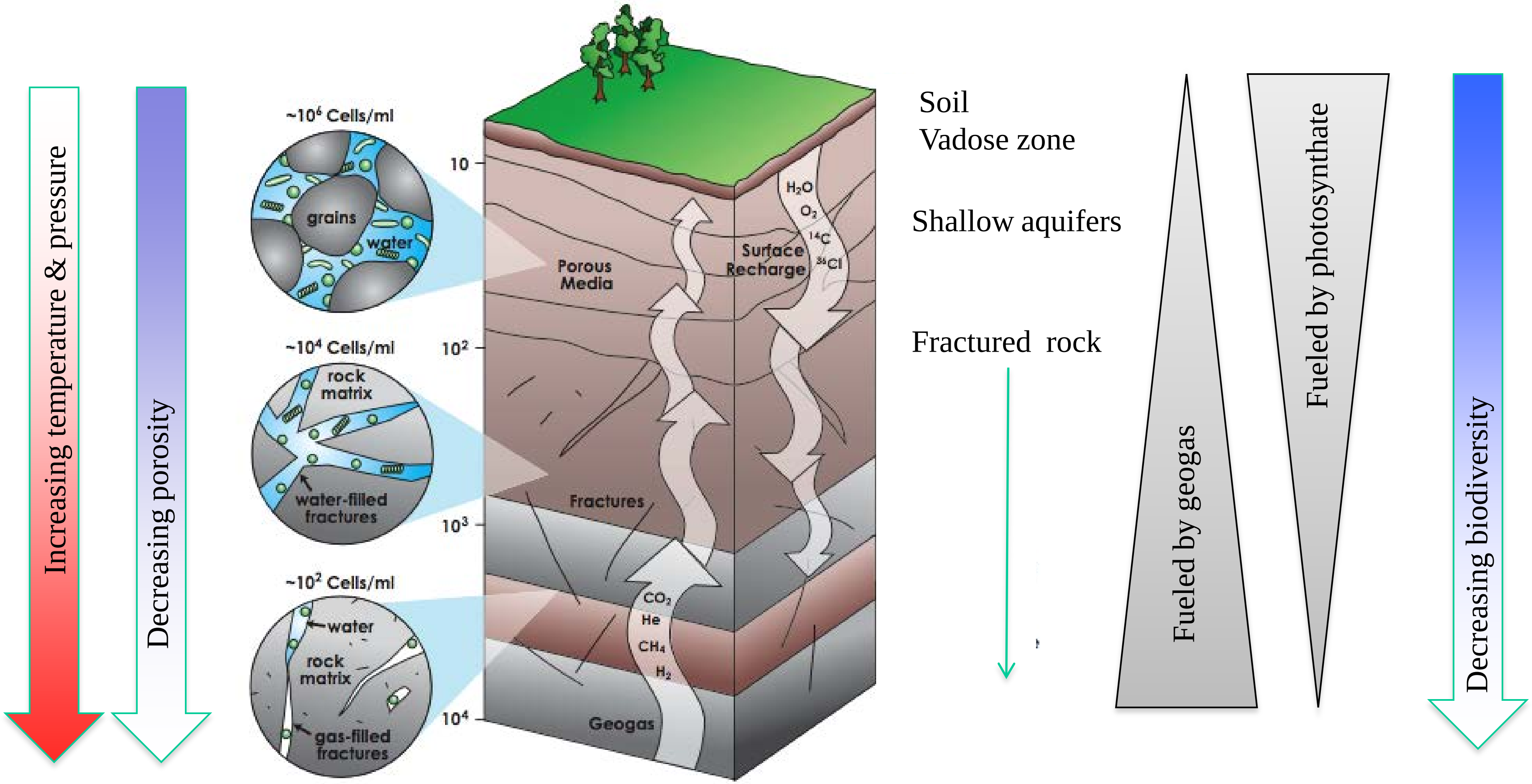
Outline

- Deep biosphere background
- Sampling methods
- South African mine project
- Deep underground labs

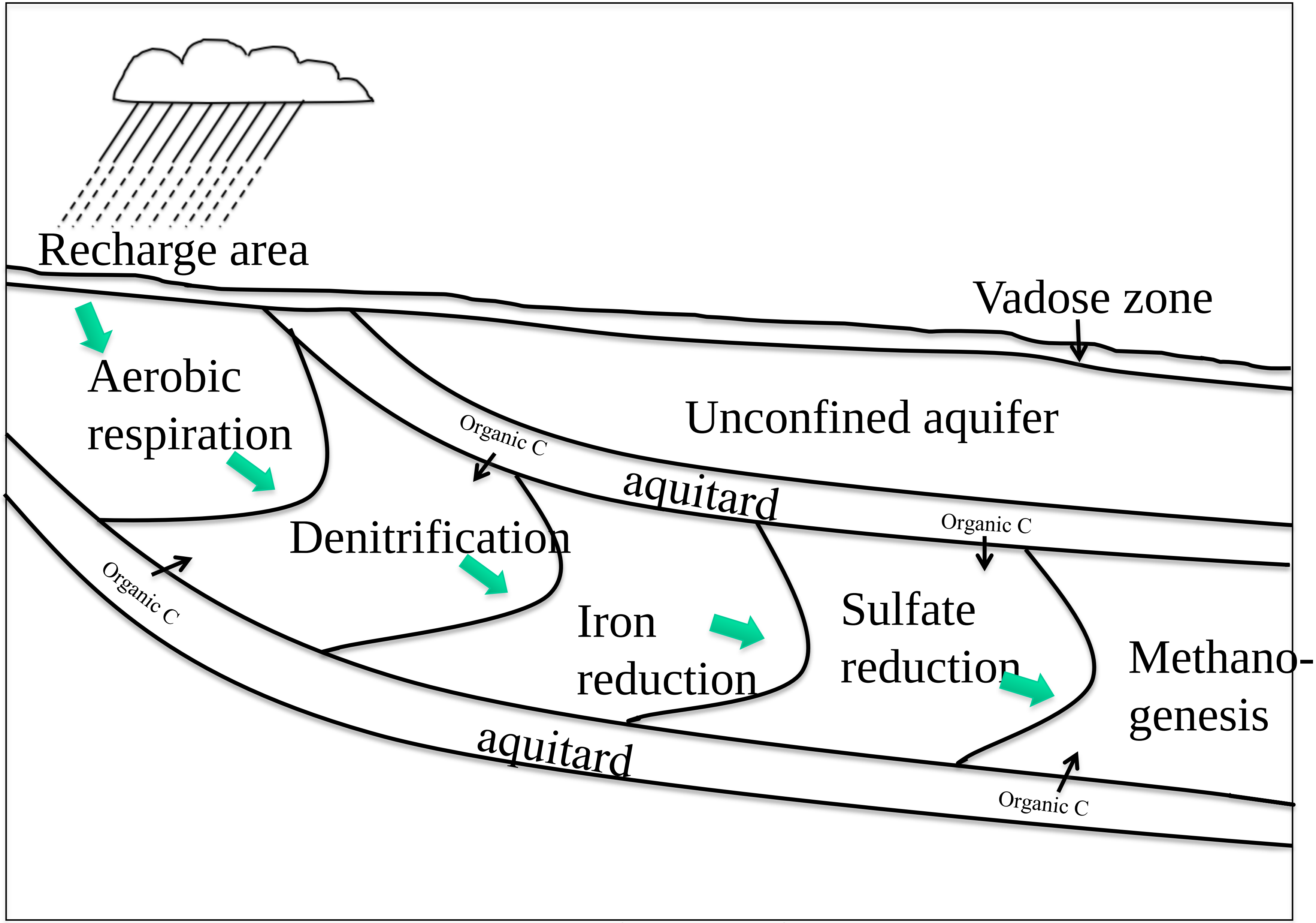
The Deep Terrestrial Biosphere -- ~25 years of research



- Funding: U.S. Dept. of Energy, Nat'l Science Foundation, NASA, others
- Major Accomplishments
  - Drilling and tracer technologies
  - Extended known biosphere to >4 km
  - Revealed biomass & biodiversity
  - Isolates in culture collections
  - Linked microbial activity with geological interfaces
  - Slow rates of subsurface microbial activity
  - Discovery of subsurface lithoautotrophic microbial ecosystems (SLiMEs)



Increasing temperature & pressure
Decreasing porosity
~10⁶ Cells/ml
grains
water
~10⁴ Cells/ml
rock matrix
water-filled fractures
~10² Cells/ml
water
rock matrix
gas-filled fractures
10
10²
10³
10⁴
Porous Media
Fractures
Geogas
Surface Recharge
H_2O
O_2
^{14}C
^{36}Cl
CO_2
He
CH_4
H_2
Soil
Vadose zone
Shallow aquifers
Fractured rock
Fueled by geogas
Fueled by photosynthate
Decreasing biodiversity



Terminal electron-accepting processes along a flow-path. After Smith and Harris (2007)

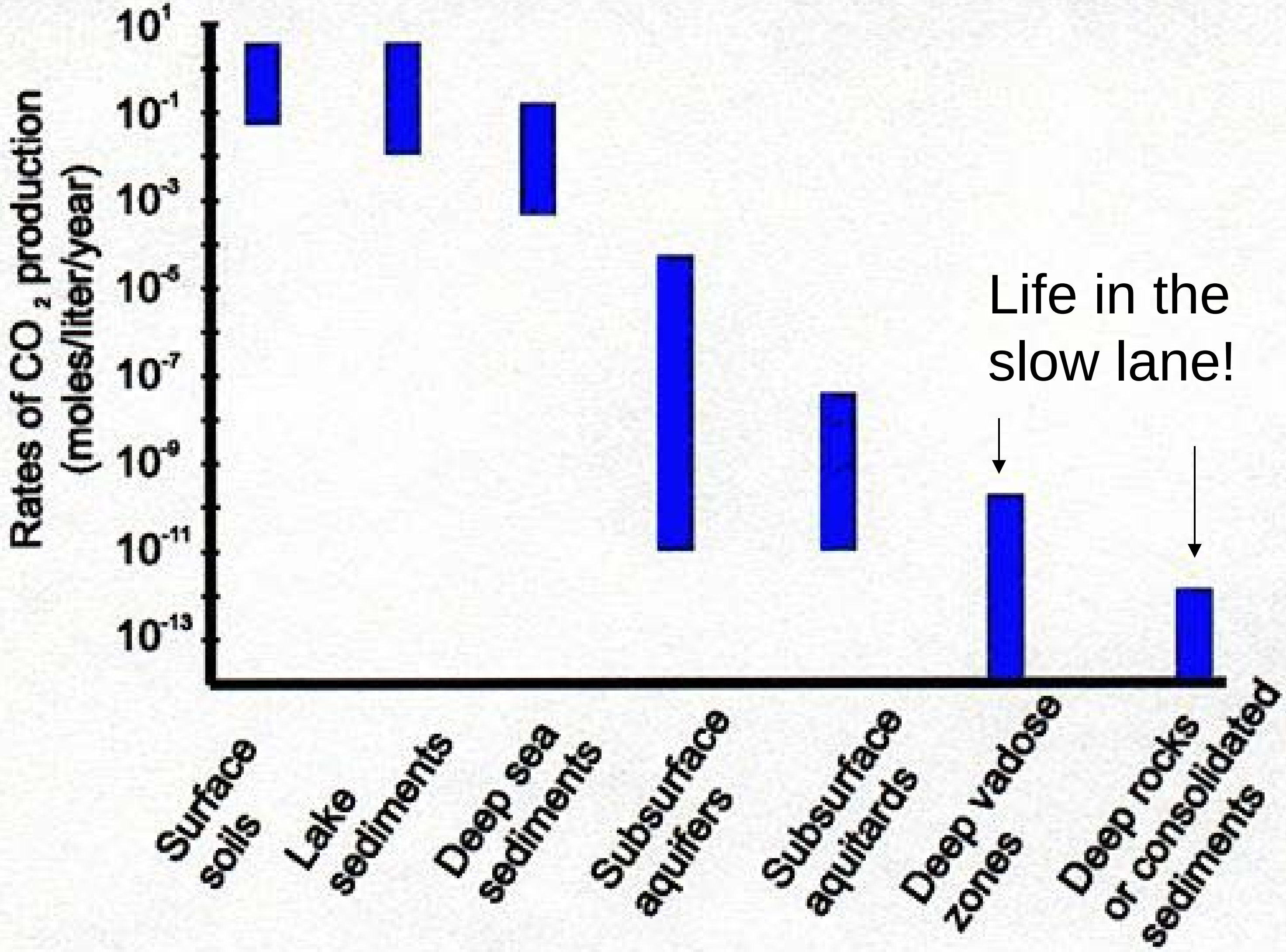
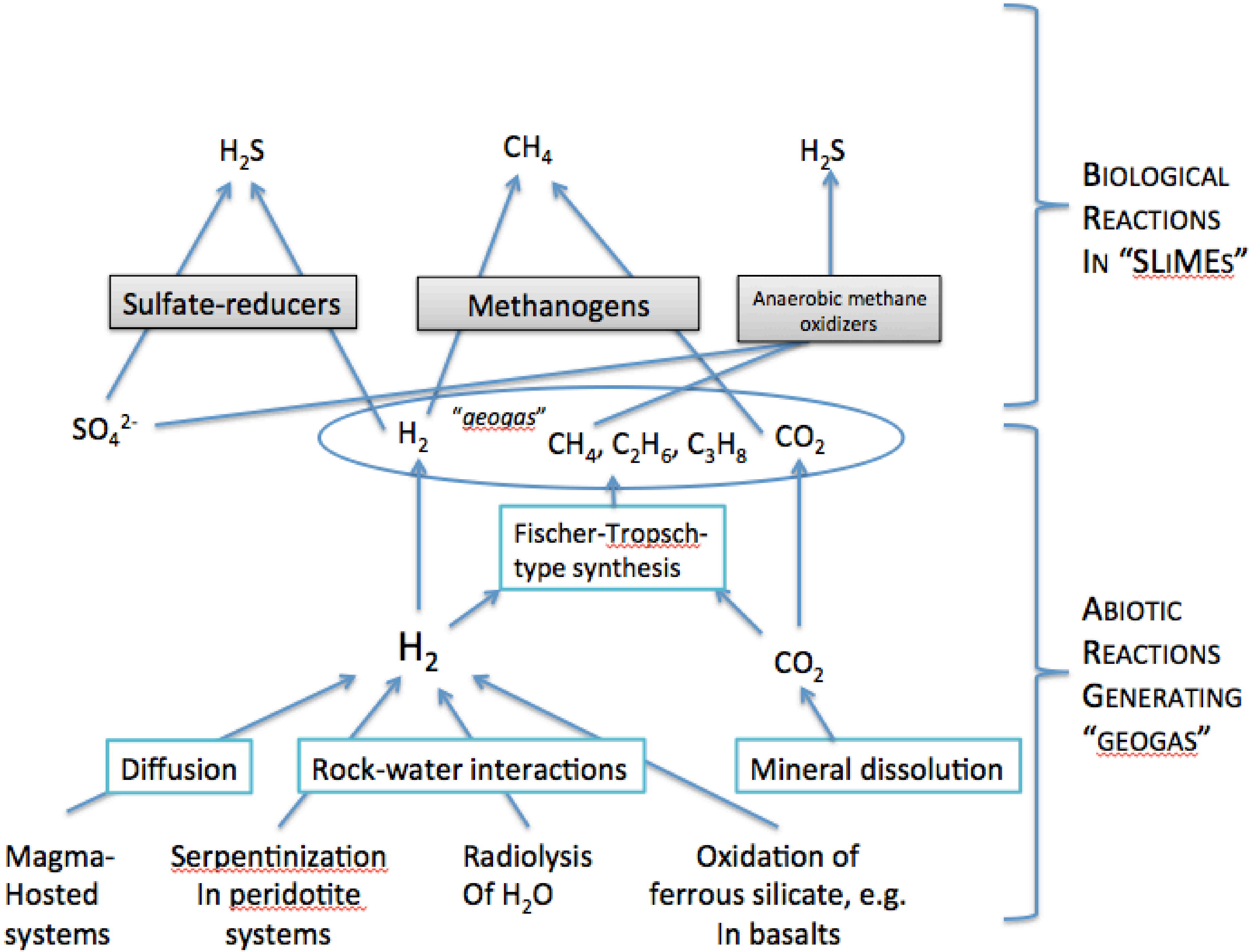


FIGURE 4.22 Ranges of rates of *in situ* CO_2 production for various surface and subsurface environments, as estimated by groundwater chemical analyses and geochemical modeling. (Adapted from Kieft and Phelps, 1997.)



H_2S
CH_4
H_2S
Sulfate-reducers
Methanogens
Anaerobic methane oxidizers
Biological Reactions In "SLiMEs"
SO_4^{2-}
"geogas"
H_2
CH_4, C_2H_6, C_3H_8
CO_2
Fischer-Tropsch-type synthesis
H_2
CO_2
Abiotic Reactions Generating "geogas"
Diffusion
Rock-water interactions
Mineral dissolution
Magma-Hosted systems
Serpentinization In peridotite systems
Radiolysis Of H_2O
Oxidation of ferrous silicate, e.g. In basalts

Deep Biosphere: Major Research Questions

(from NSF Deep Science)

1. How deeply does life extend into the Earth?
2. What fuels the deep biosphere?
3. How does the interplay between biology and geology shape the subsurface?
4. What are subsurface genomes telling us?
5. Did life on the earth's surface come from underground?
6. Is there life as we don't know it?

1. How deeply does life extend into the Earth?

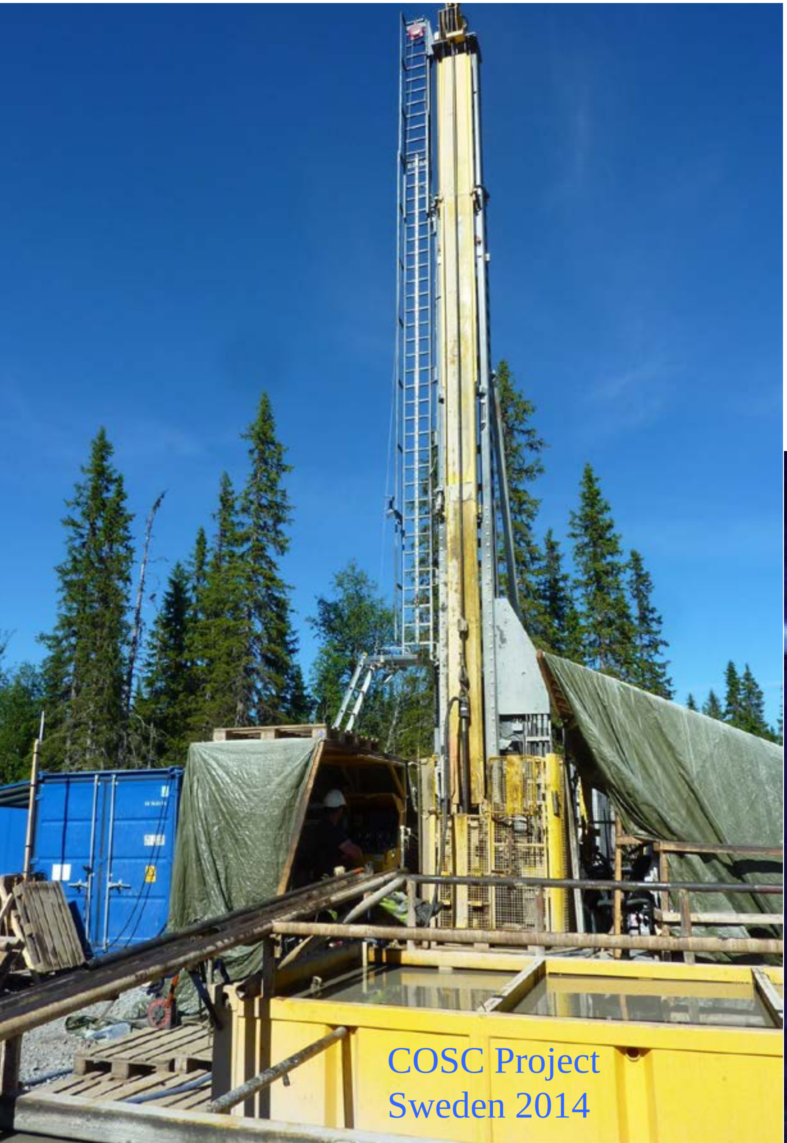
What are the factors that limit life in the deep subsurface?

- Temperature?
  - ~121° C current known upper limit for life grown in the lab.
  - Geothermal gradient: 10-60° C/km, average ~20° C/km.
  - Temperature-limited biosphere may extend 2-12 km
  - Petroleum reservoirs: microbes decline sharply at 80-90° C. Then what else limits life?
- Pressure? Energy and nutrient availability? Pore space and connectivity?
- A combination of factors?

2. What fuels the deep biosphere?

- Transported or buried organic C (photosynthate) from the surface.
- Rock-water interactions generate "Geogas" (H_2, hydrocarbons)
  - Basalt-water interactions, e.g., Snake River Plain basalt aquifers (Stevens and McKinley, 1995; Chapelle et al., 2002)
  - Serpentinization, e.g., at the Lost City vent (Kelley et al., 2005)
  - Granite-water interactions, Aspo Hard Rock Lab, Sweden (Pedersen, 1997)
  - Radiolysis of water, e.g., Witwatersrand Basin, South Africa (Lin et al., 2005, 2006).

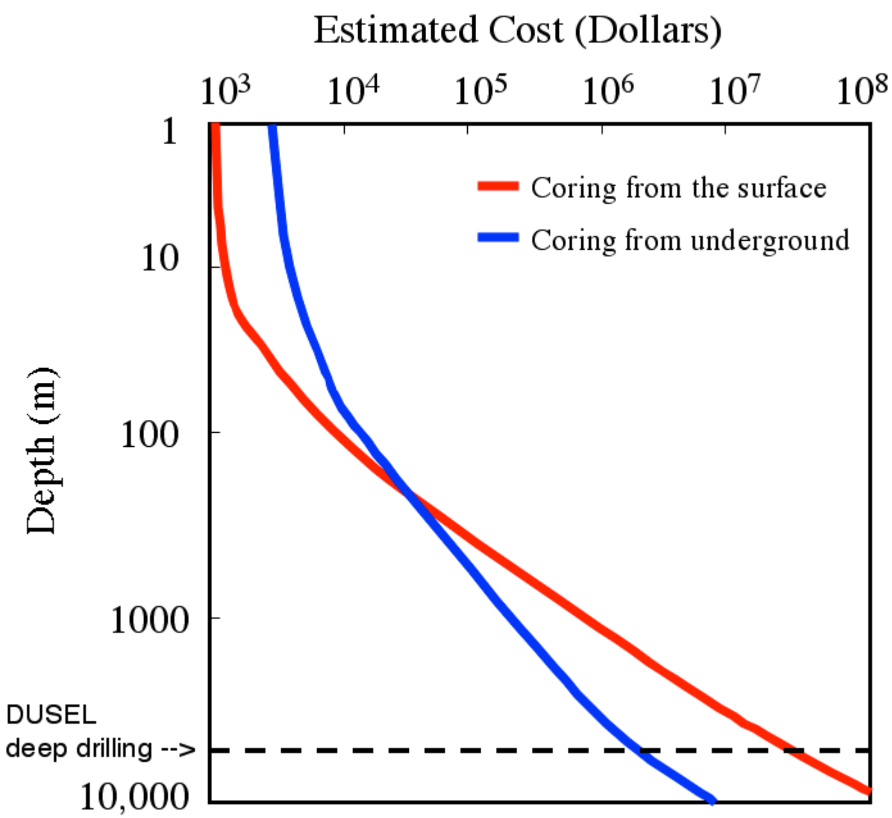
Sampling the deep biosphere

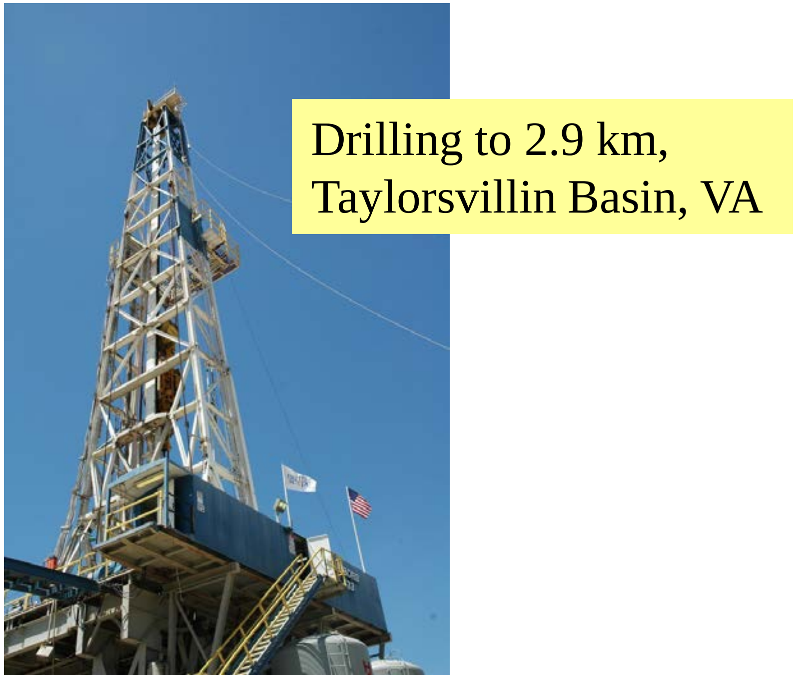


- Drilling/coring from the surface

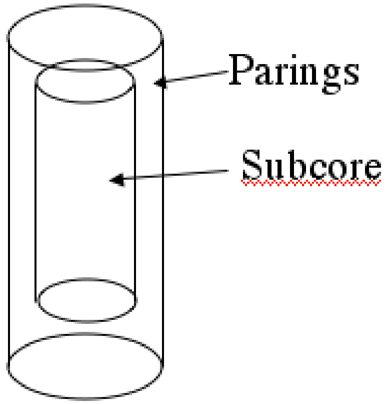
- Access via deep mines or underground labs

Drilling to great depth is cheaper if you start in a deep mine





Subcoring and tracers:



- tracers
  - Solute: Br^-, fluorochromes (e.g., rhodamine), perfluorinated hydrocarbons
  - Particulate: fluorescent carboxylated 1-µm microbeads
- core diameters ≥2 inches preferred
- drilling methods are highly site specific.
- anaerobic glove bag
- core barrels should be steam cleaned, core barrel liners

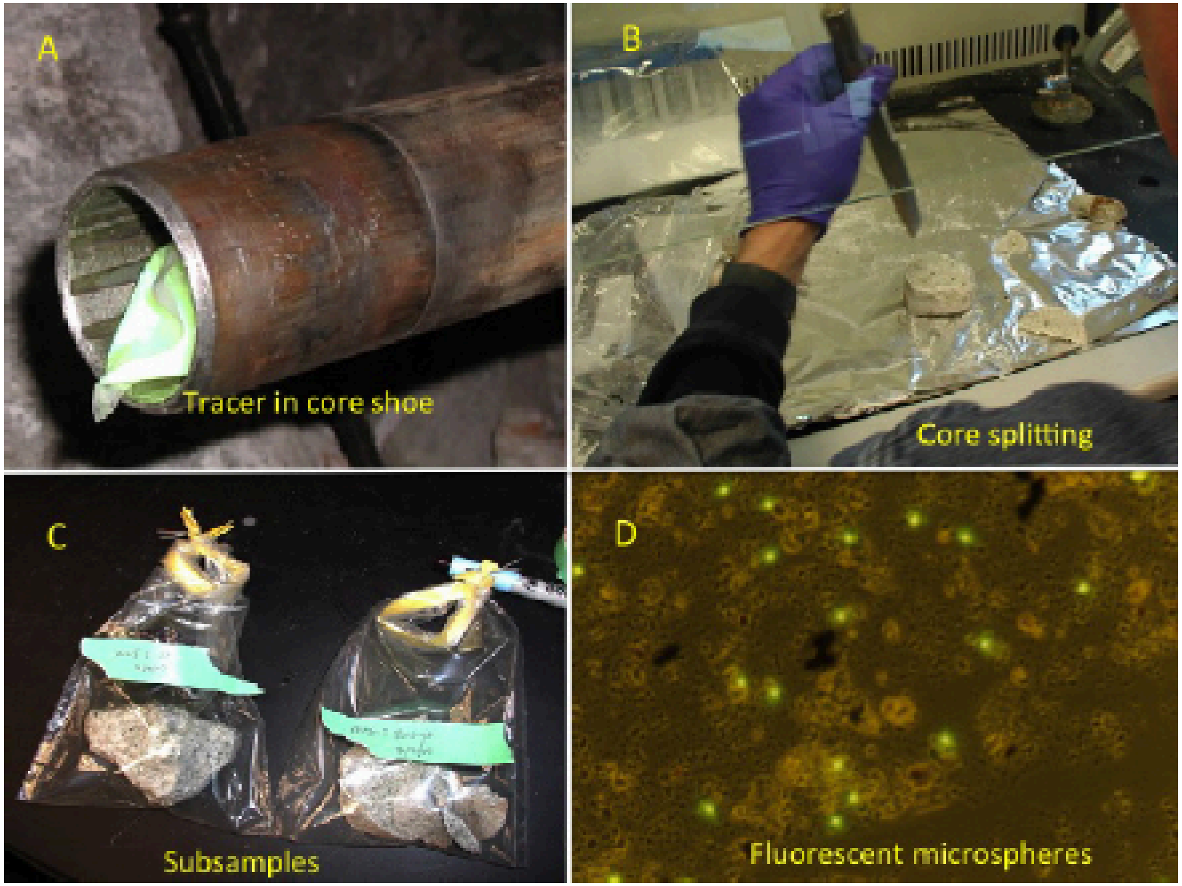


Figure 3. Use of tracers and subcoring for geomicrobiological sampling in a granitic subsurface environment (Sahl et al., 2008). **(a)** Fluorescent microbead tracers deployed in the core shoe, **(b)** subcoring using a hammer and chisel in a laminar flow hood, **(c)** subcore samples in Whirl-Pak® bags, and **(d)** fluorescent microbead tracers in drilling mud, viewed by epifluorescence microscopy.

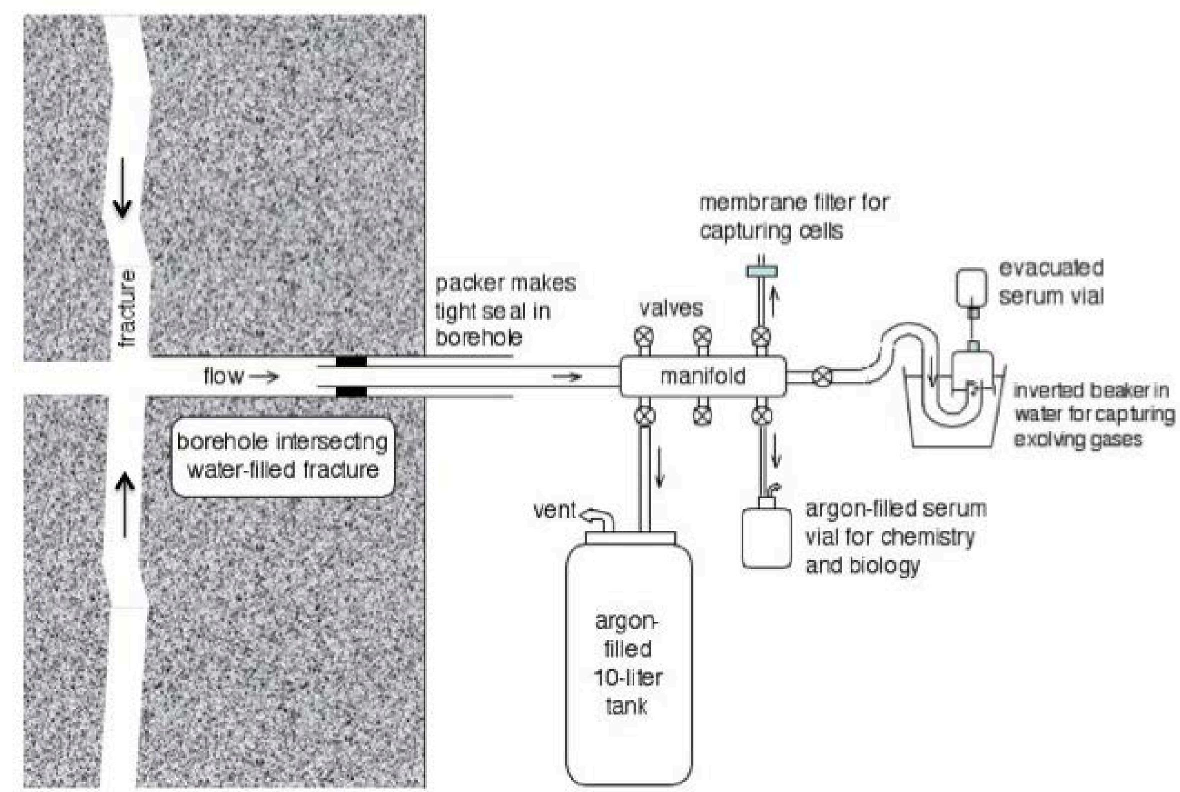
Kieft et al. 2015. *Scientific Drilling* 19:43–53

Sampling in deep mines & underground labs

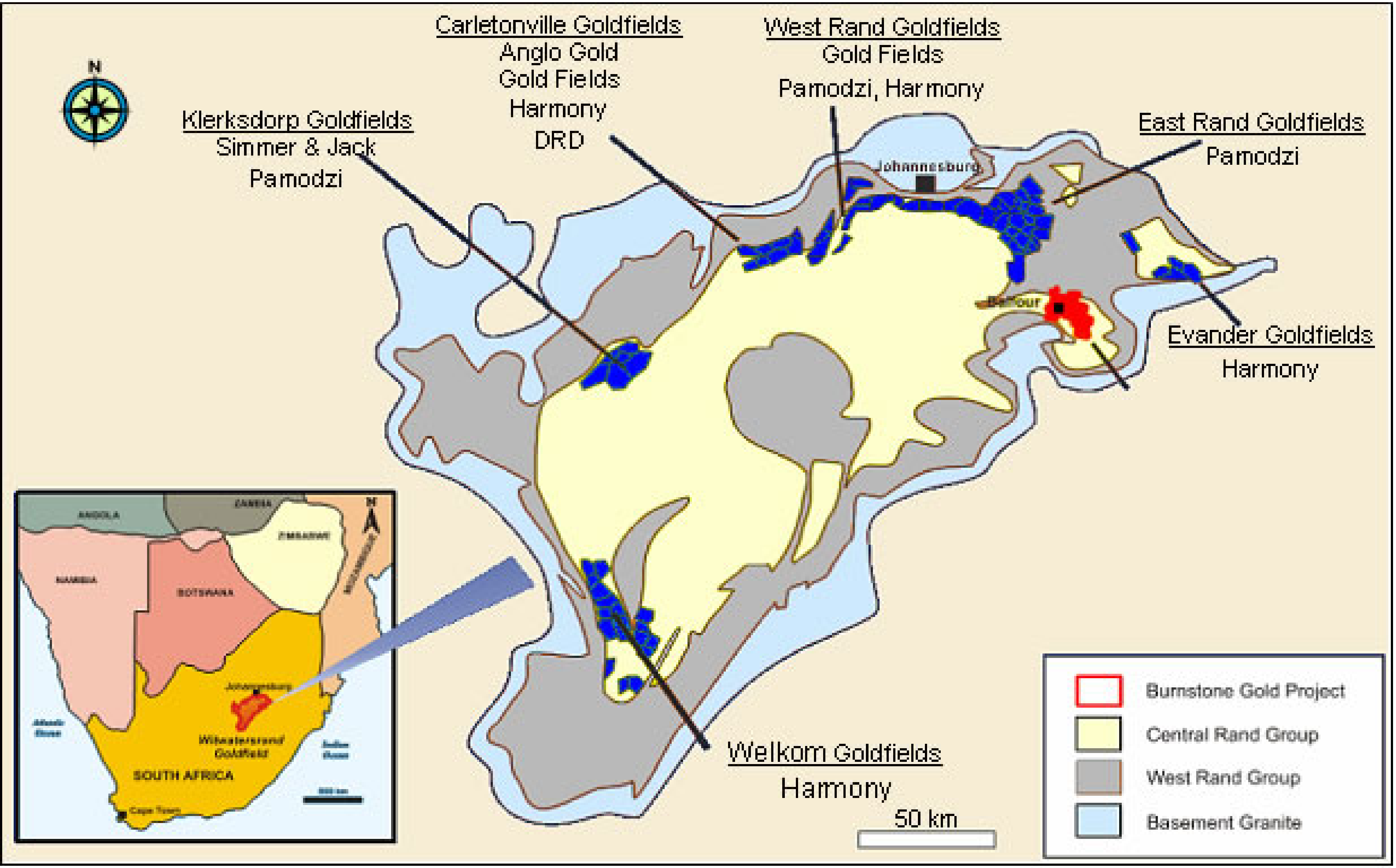
- Rocks from freshly mined surfaces
- **Fissure water from flowing boreholes**
  - Filtered to concentrate cells
    - including massive filtering (~10,000 liters)
  - In situ enrichment devices
- Cores -- especially useful for sampling rock matrix, fractures
- Biofilms

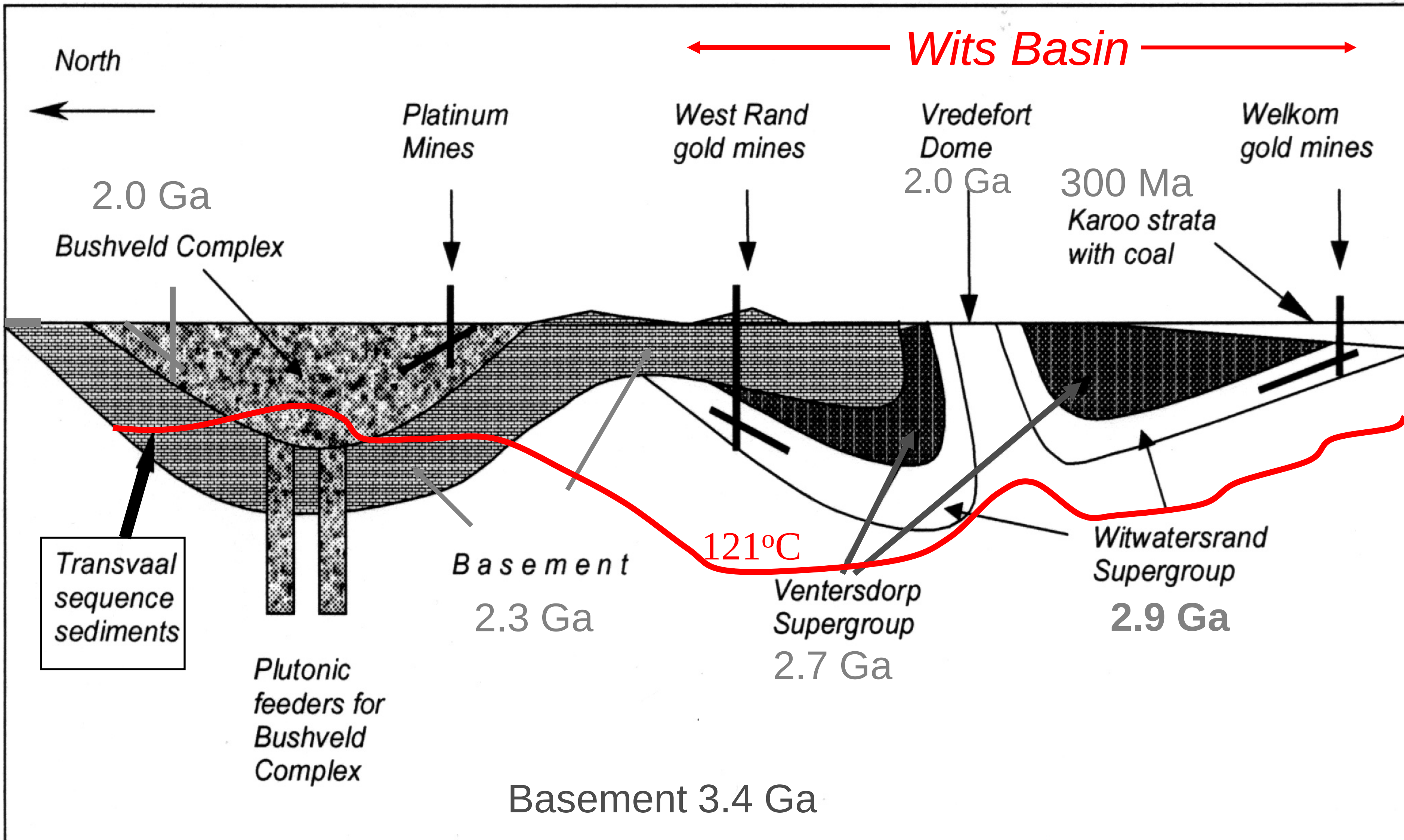
Drilling a borehole and sampling groundwater
at 3 km depth in a South African gold mine.

Sampling deep, ancient fracture water in mines



Witwatersrand Deep Microbiology Project



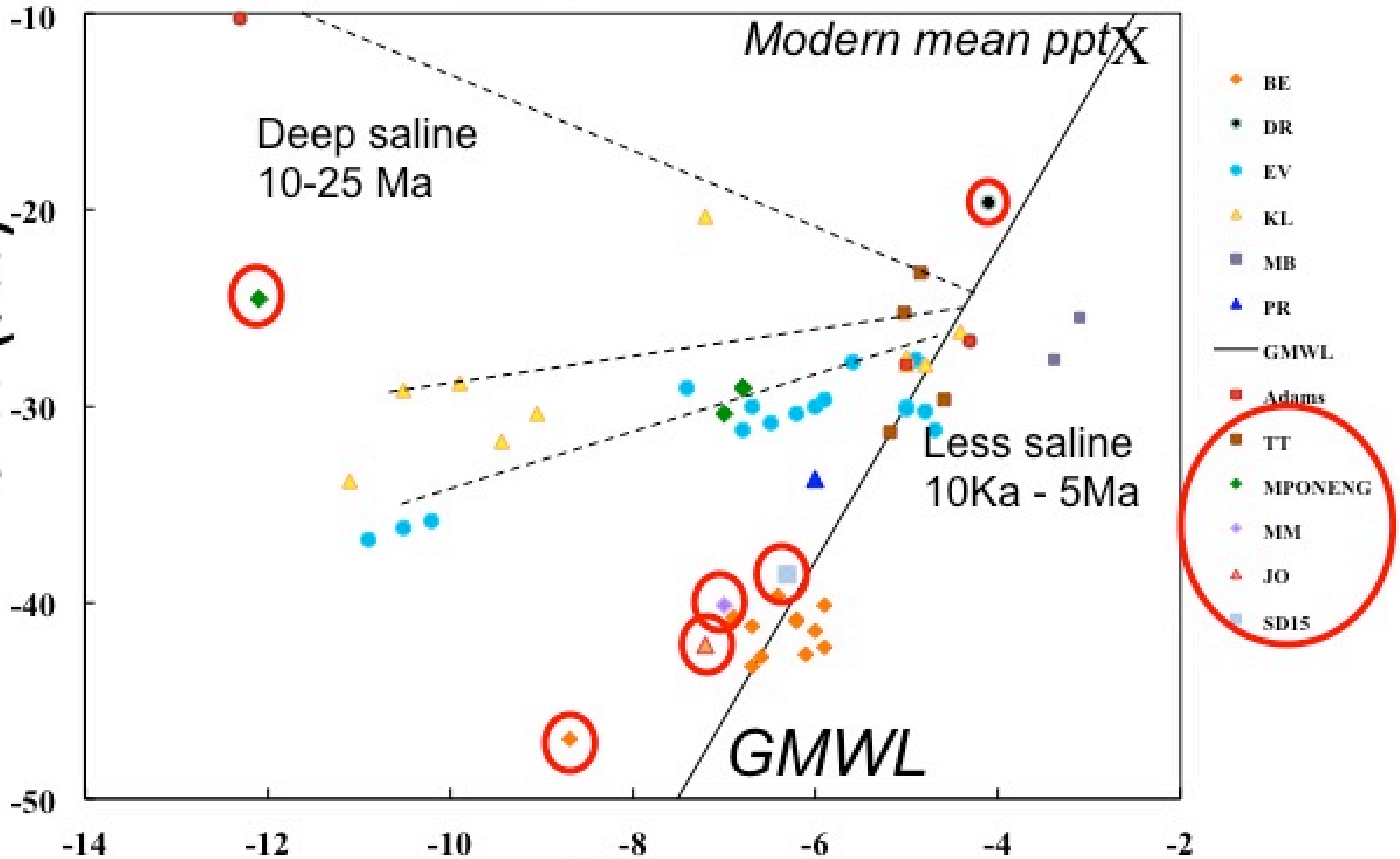


Geothermal gradients:

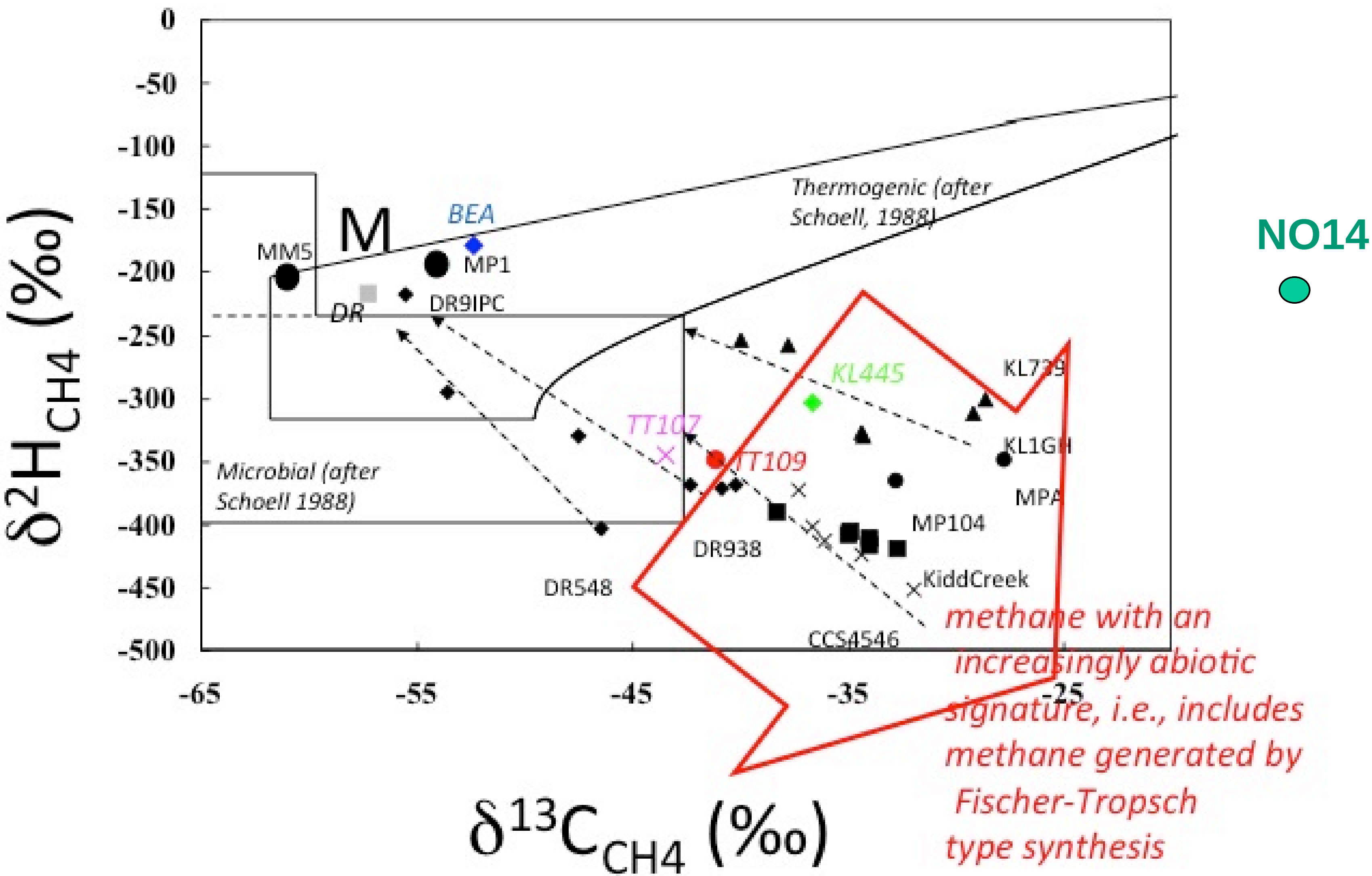
25ºC/km 9-15ºC/km 20ºC/km

δ^2H vs. $\delta^{18}O$

Two geologically old end-members

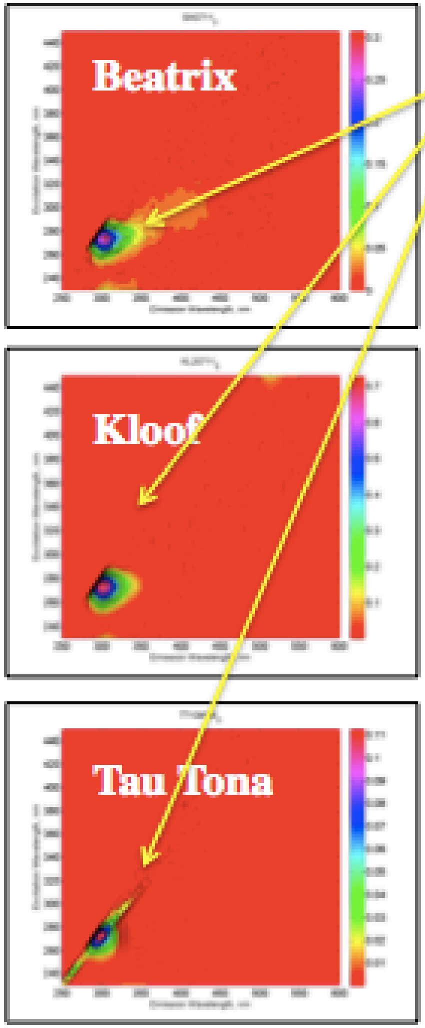


Barbara Sherwood Lollar


NO14
0
-50
-100
-150
-200
-250
-300
-350
-400
-450
-500
-65
-55
-45
-35
-25
δ^2H_{CH4} (‰)
$\delta^{13}C_{CH4}$ (‰)
M
BEA
MM5
MP1
DR
DR9IPC
Thermogenic (after Schoell, 1988)
KL445
KL739
TT107
TT109
KL1GH
MPA
MP104
Microbial (after Schoell 1988)
DR938
DR548
KiddCreek
CCS4546
methane with an increasingly abiotic signature, i.e., includes methane generated by Fischer-Tropsch type synthesis

DOC Characterization

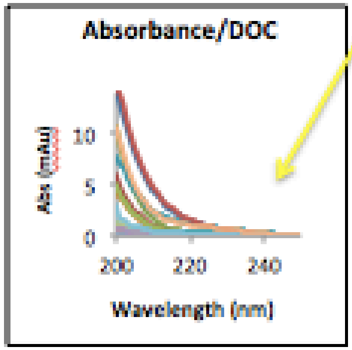
(0.05-0.2 mM)



Excitation-emission matrices (EEMs):

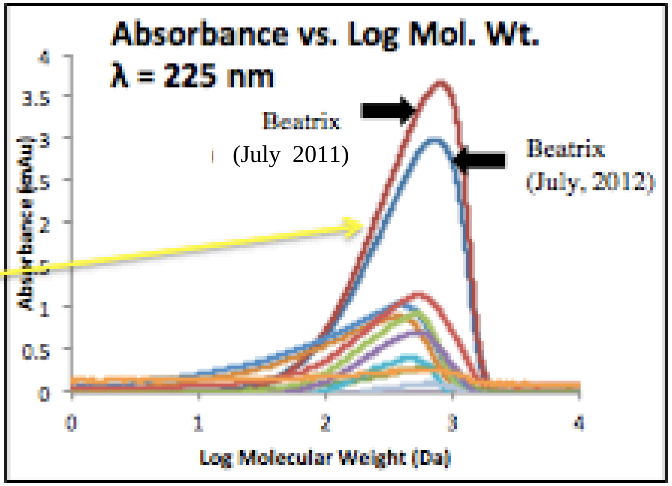
most samples exhibit a peak at $Ex_{\lambda}/Em_{\lambda}$ = 275 nm/ 300 nm

Probable protein-like (tyr and phe) peaks



No absorbance over 240 nm:

Little to no aromatic content



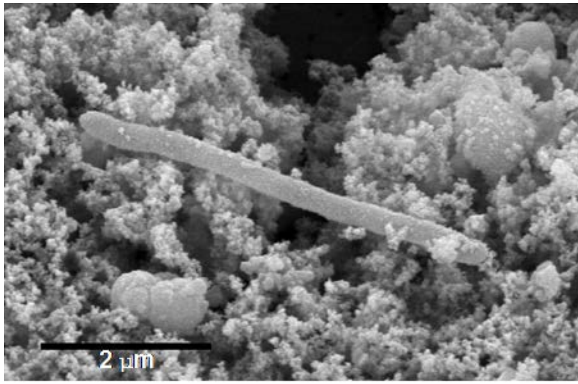
Molecular weight distribution, HPLC size exclusion:

10 amu < MW < 2000 Da

Mode: ~ 800 Da

GC-MS and solid-state **NMR** in progress

Mike Pullin and Sarah Hendrickson, NMT



Desulforudis audaxviator

Ubiquitous in 3-km deep groundwater

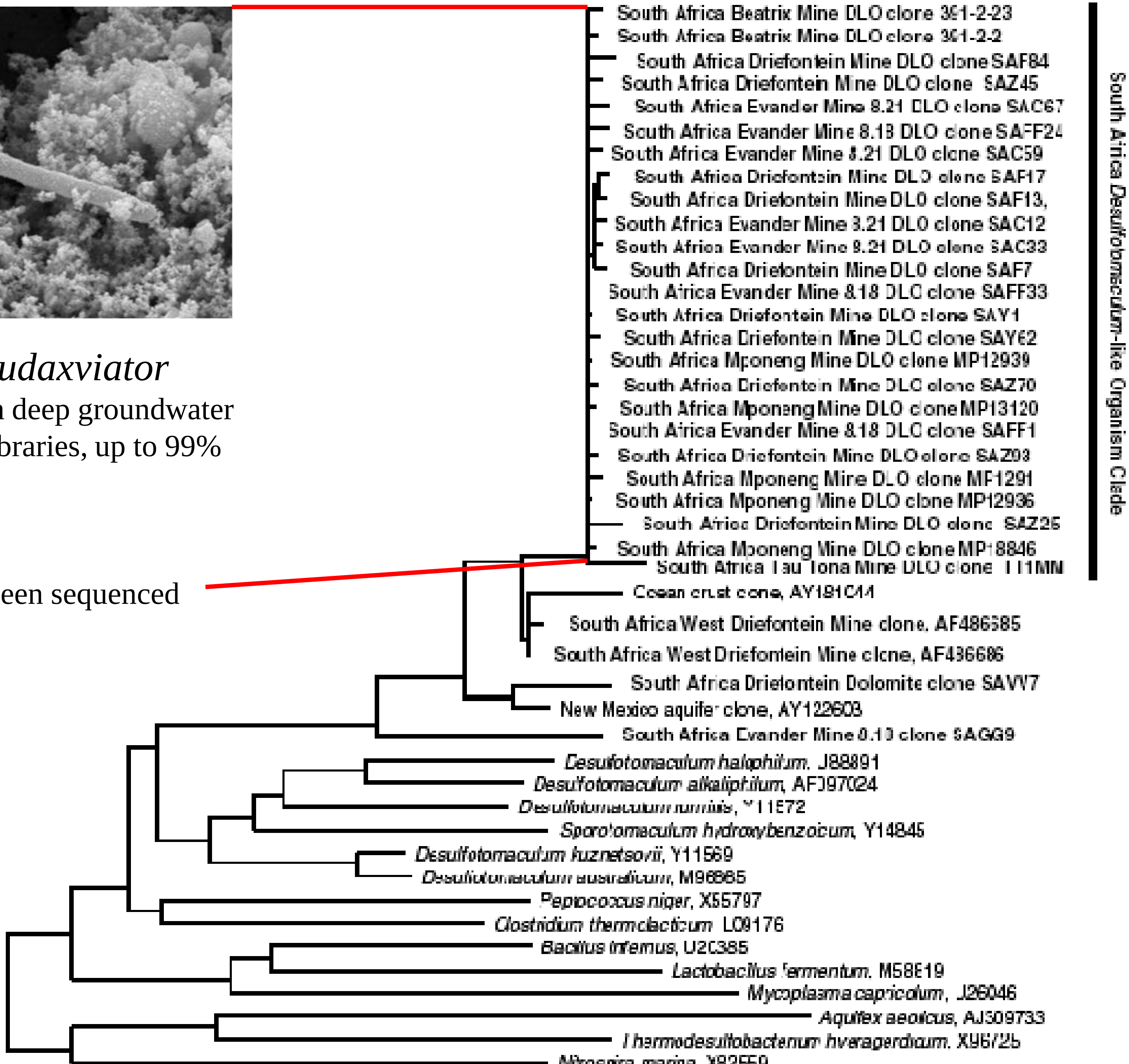
Dominates clone libraries, up to 99%

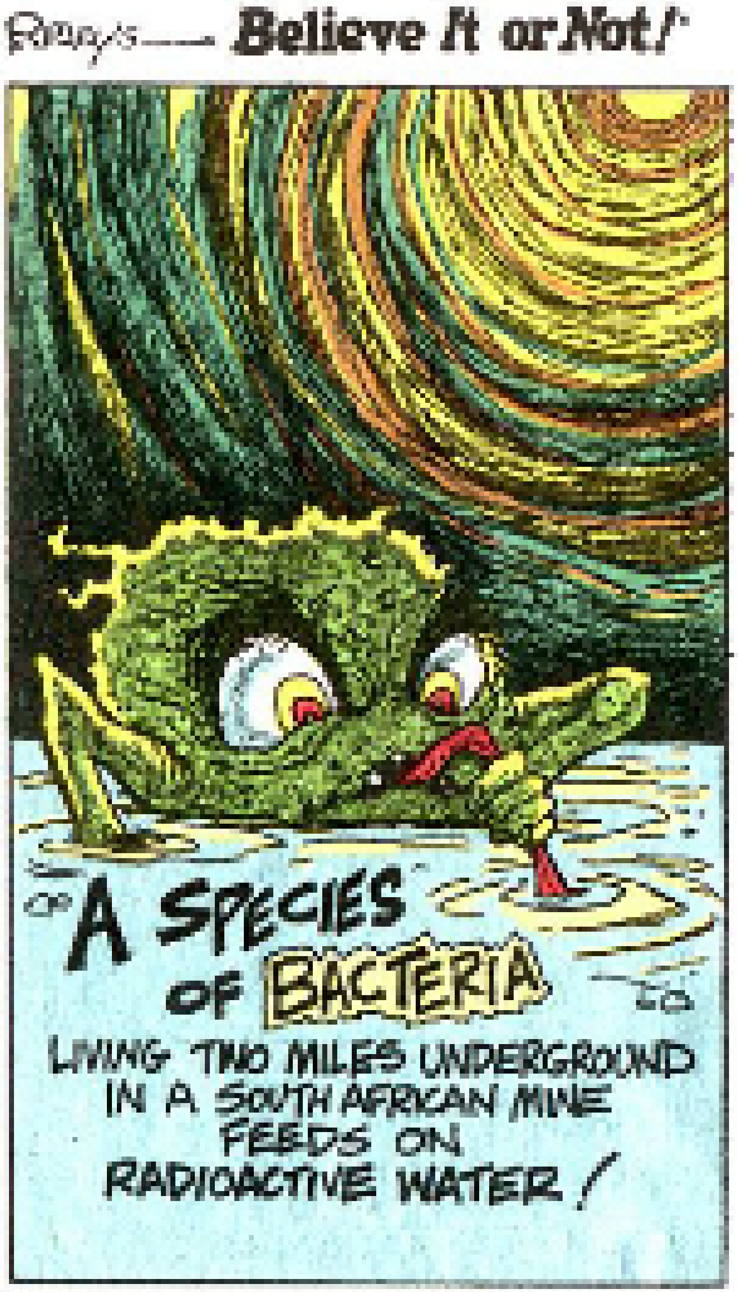
Sulfate-reducer

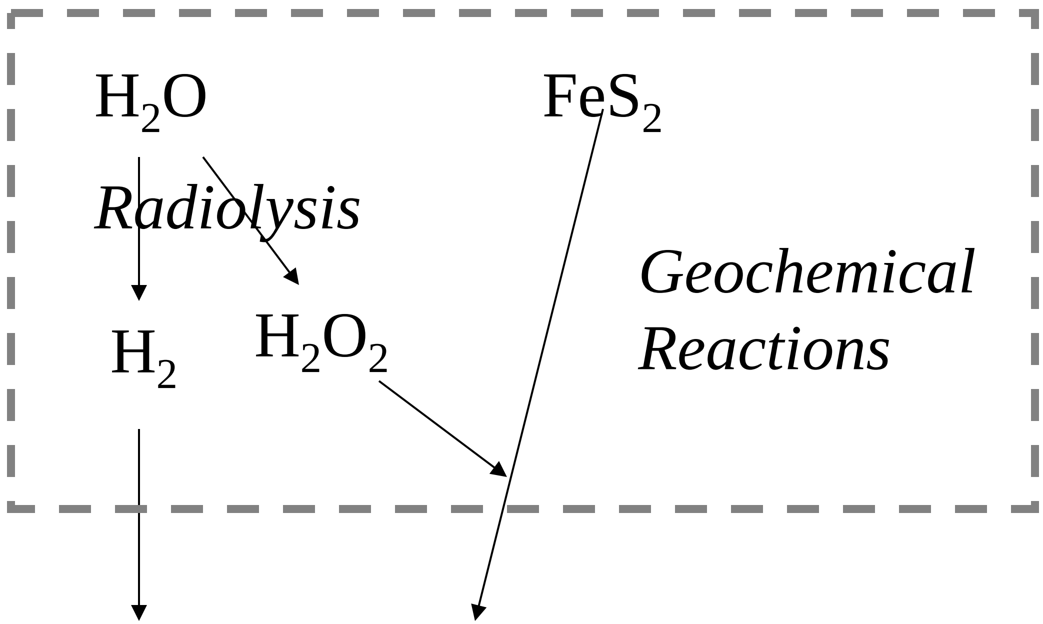
Novel taxon

Uncultured

Genome has now been sequenced

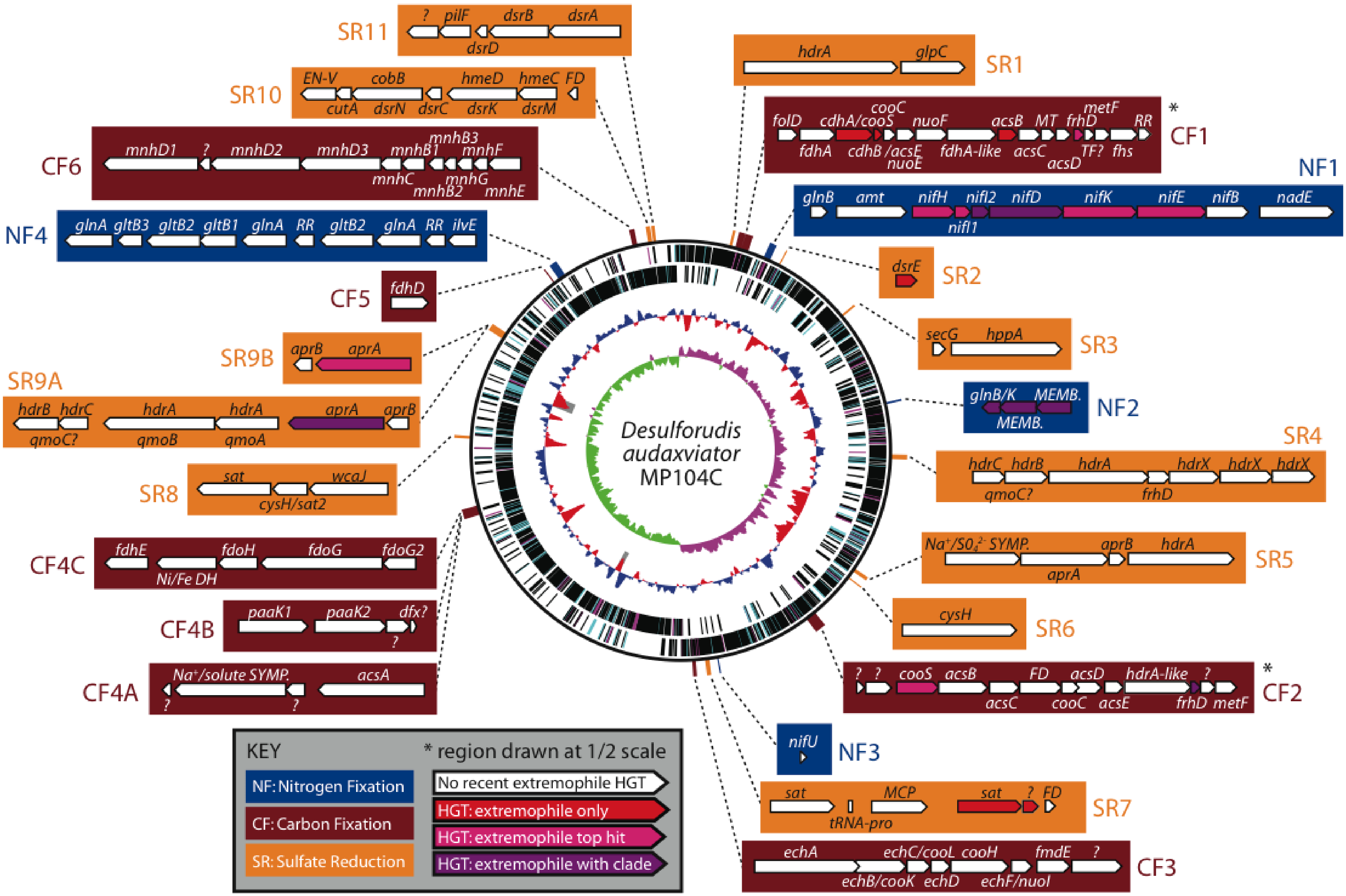


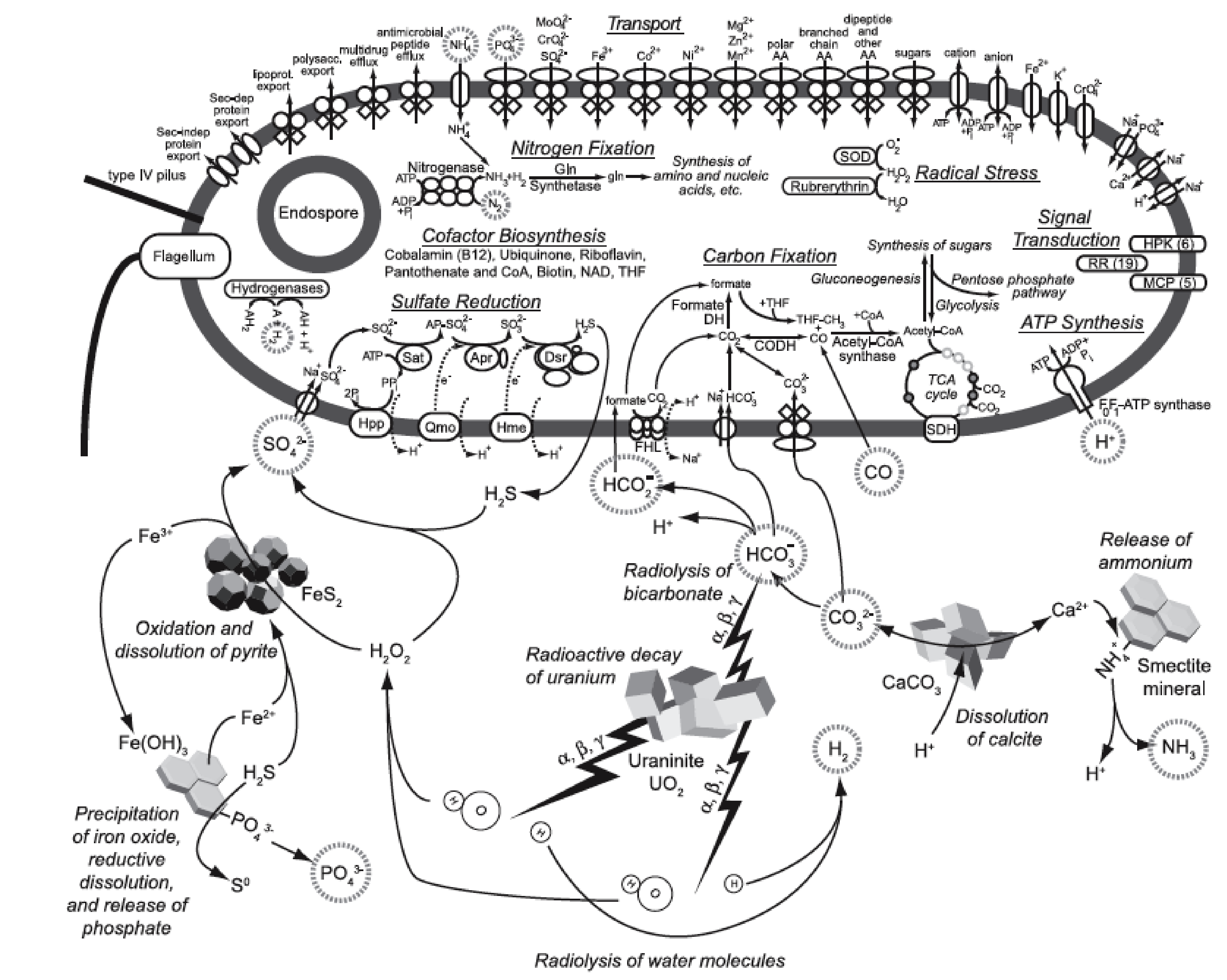




$$4H_2 + H^+ + SO_4^{2-} \xrightarrow{SRB} HS^- + 4H_2O$$

Desulforudis audaxviator


SR11
? pilF dsrB dsrA
dsrD
SR10
EN-V cobB hmeD hmeC FD
cutA dsrN dsrC dsrK dsrM
CF6
mnhD1 ? mnhD2 mnhD3 mnhB1 mnhB3 mnhF
mnhC mnhG mnhB2 mnhE
NF4
glnA gltB3 gltB2 gltB1 glnA RR gltB2 glnA RR ilvE
CF5
fdhD
SR9B
aprB aprA
SR9A
hdrB hdrC hdrA hdrA aprA aprB
qmoC? qmoB qmoA
SR8
sat wcaJ
cysH/sat2
CF4C
fdhE fdoH fdoG fdoG2
Ni/Fe DH
CF4B
paaK1 paaK2 dfx?
?
CF4A
Na⁺/solute SYMP. acsA
? ?
SR1
hdrA glpC
CF1 *
folD cdhA/cooS cooC nuoF acsB MT frhD metF RR
fdhA cdhB/acsE nuoE fdhA-like acsC acsD TF? fhs
NF1
glnB amt nifH nifI2 nifD nifK nifE nifB nadE
nifI1
SR2
dsrE
SR3
secG hppA
NF2
glnB/K MEMB.
MEMB.
SR4
hdrC hdrB hdrA hdrX hdrX hdrX
qmoC? frhD
SR5
Na⁺/SO₄²⁻ SYMP. aprB hdrA
aprA
SR6
cysH
CF2 *
? ? cooS acsB FD acsD hdrA-like ?
acsC cooC acsE frhD metF
NF3
nifU
SR7
sat MCP sat ? FD
tRNA-pro
CF3
echA echC/cooL cooH fmdE ?
echB/cooK echD echF/nuoI
Desulforudis audaxviator MP104C
KEY
* region drawn at 1/2 scale
NF: Nitrogen Fixation
CF: Carbon Fixation
SR: Sulfate Reduction
No recent extremophile HGT
HGT: extremophile only
HGT: extremophile top hit
HGT: extremophile with clade


Transport
antimicrobial peptide efflux
multidrug efflux
polysacc. export
lipoprot. export
Sec-dep protein export
Sec-indep protein export
type IV pilus
NH_4^+
PO_4^{3-}
MoO_4^{2-} CrO_4^{2-} SO_4^{2-}
Fe^{3+}
Co^{2+}
Ni^{2+}
Mg^{2+} Zn^{2+} Mn^{2+}
polar AA
branched chain AA
dipeptide and other AA
sugars
cation
anion
Fe^{2+}
K^+
CrO_4^{2-}
ATP
ADP +P_i
Na^+ PO_4^{3-}
Na^+
Ca^{2+}
H^+
Nitrogen Fixation
Nitrogenase
ATP
ADP +P_i
NH_3+H_2
N_2
Gln Synthetase
gln
Synthesis of amino and nucleic acids, etc.
SOD
Rubrerythrin
O_2^-
H_2O_2
H_2O
Radical Stress
Endospore
Flagellum
Cofactor Biosynthesis
Cobalamin (B12), Ubiquinone, Riboflavin, Pantothenate and CoA, Biotin, NAD, THF
Hydrogenases
AH_2
$A + H_2$
$AH + H^+$
Sulfate Reduction
SO_4^{2-}
$AP-SO_4^{2-}$
SO_3^{2-}
H_2S
ATP
Sat
Apr
Dsr
PP
2P
e^-
Hpp
Qmo
Hme
H^+
Na^+ SO_4^{2-}
Carbon Fixation
formate
Formate DH
+THF
THF-CH_3
+CoA
Acetyl-CoA
CO_2
CODH
CO
Acetyl-CoA synthase
CO_3^{2-}
formate CO_2
FHL
Na^+
Na^+ HCO_3^-
Synthesis of sugars
Gluconeogenesis
Pentose phosphate pathway
Glycolysis
TCA cycle
CO_2
SDH
Signal Transduction
HPK (6)
RR (19)
MCP (5)
ATP Synthesis
ATP
ADP+ P_i
F_0F_1-ATP synthase
H^+
SO_4^{2-}
H_2S
HCO_2^-
CO
H^+
HCO_3^-
Radiolysis of bicarbonate
Fe^{3+}
FeS_2
Oxidation and dissolution of pyrite
H_2O_2
α, β, γ
Radioactive decay of uranium
CO_3^{2-}
CaCO$_3$
Ca^{2+}
Release of ammonium
NH_4^+
Smectite mineral
Dissolution of calcite
H^+
H_2
NH_3
Fe^{2+}
Fe(OH)$_3$
H_2S
Uraninite UO_2
PO_4^{3-}
S^0
Precipitation of iron oxide, reductive dissolution, and release of phosphate
H
O
Radiolysis of water molecules

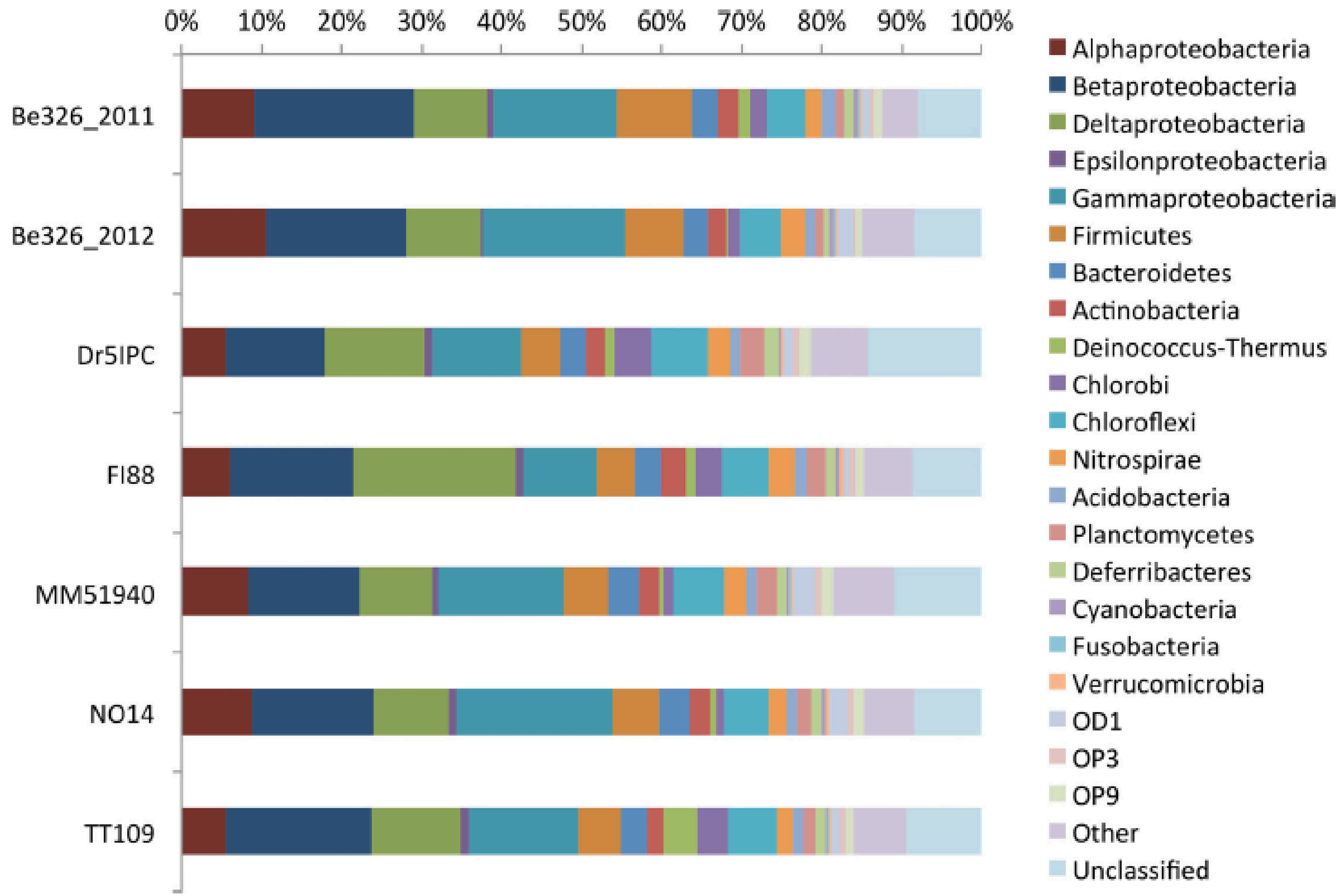


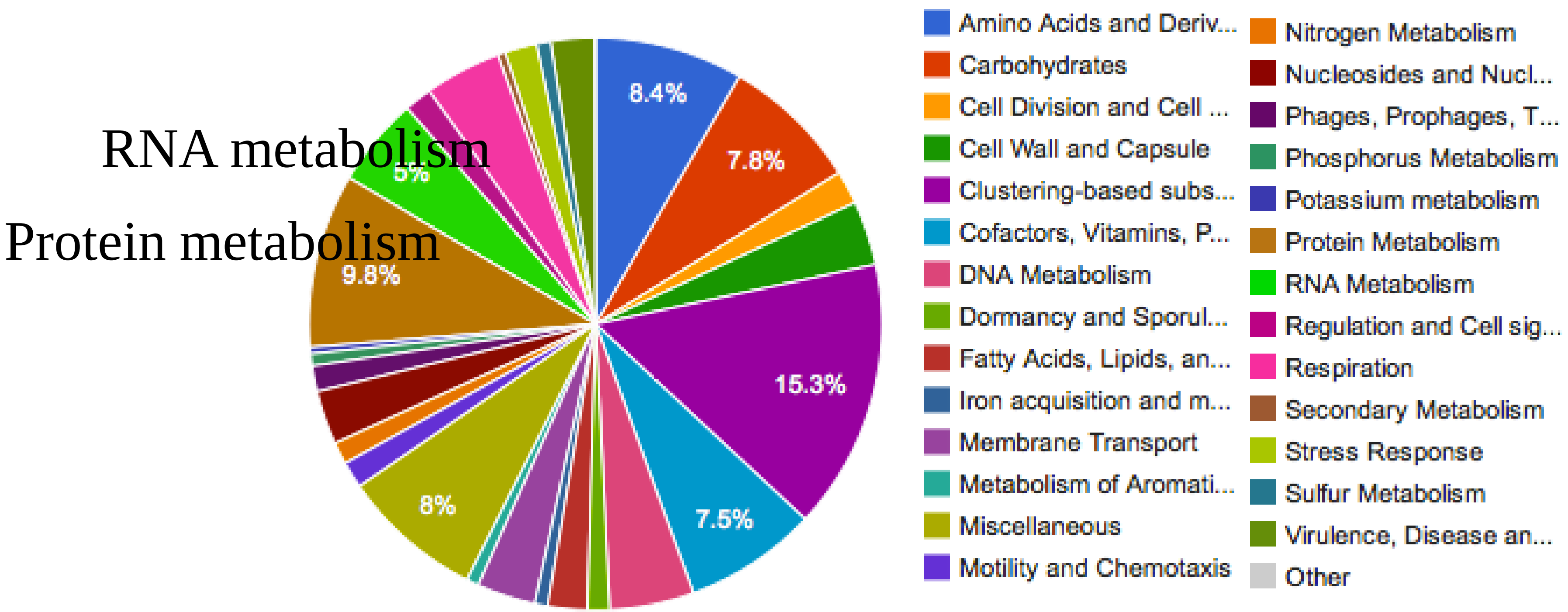
FIGURE 3 | Subsurface taxonomic distribution. A bar plot of the relative abundance (x-axis) of various phyla (color) per subsurface site (y-axis). Due to its high relative abundance, the phylum proteobacteria was split into its corresponding classes. Members of the "Other" bin include: BRC1, Caldiserica, Chlamydiae, Chloroplast, Crenarchaeota, Dictyoglomi, Elusimicrobia, Euryarchaeota, Fibrobacteres, Gemmatimonadetes, Lentisphaerae, Mitochondria, OP1, OP2, OP8, OP10, OP11, Spirochaetes, Synergistetes, TA06, Tenericutes, TG-1, Thermotogae, TM6, TM7, WS1, WS3, WS6, and Zetaproteobacteria.

Magnabosco et al., 2014. *Front. Microbiol.* doi: 10.3389/fmicb.2014.00679

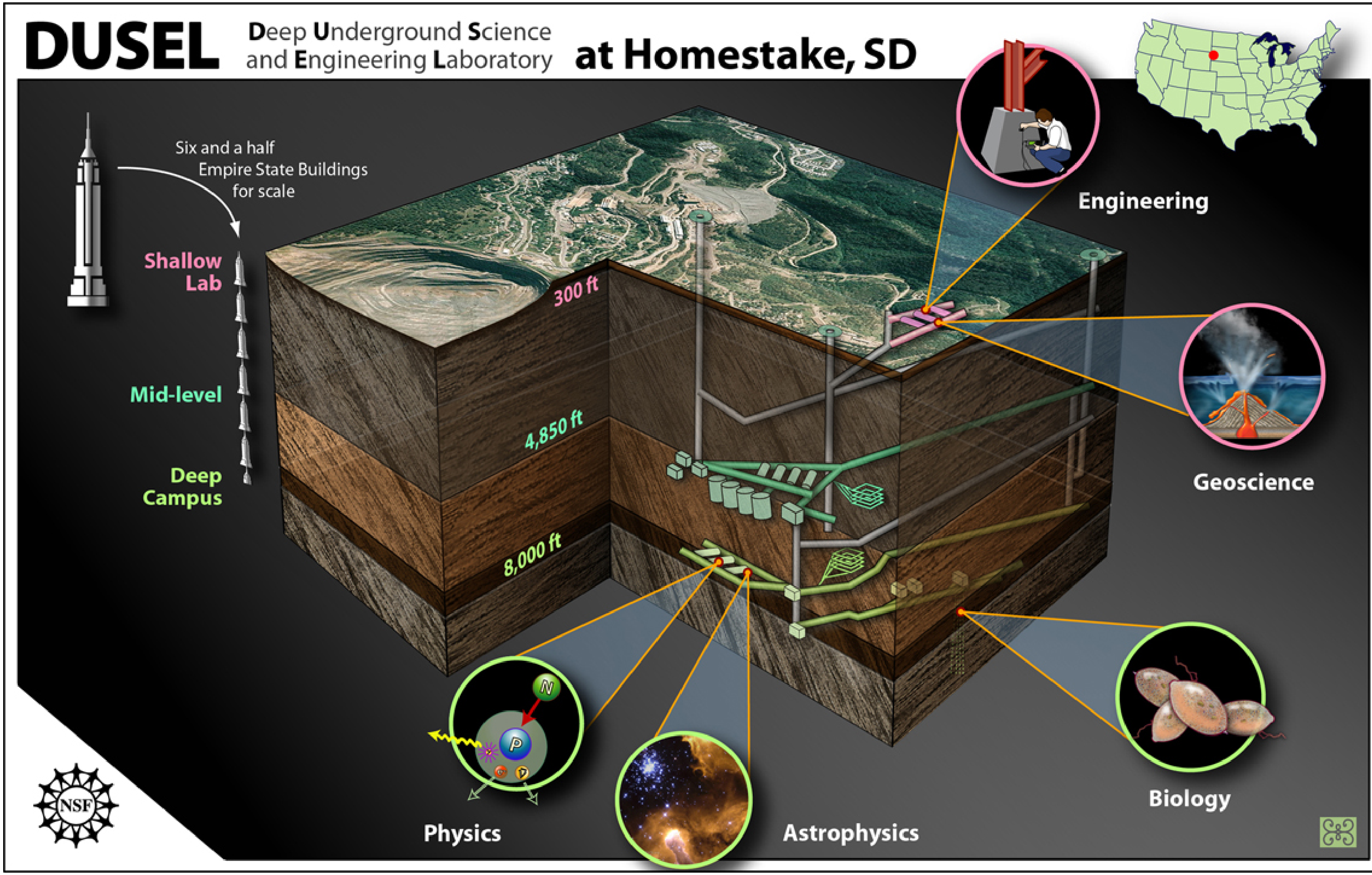
TT107FW240811

Metagenomics: Functional hierarchy

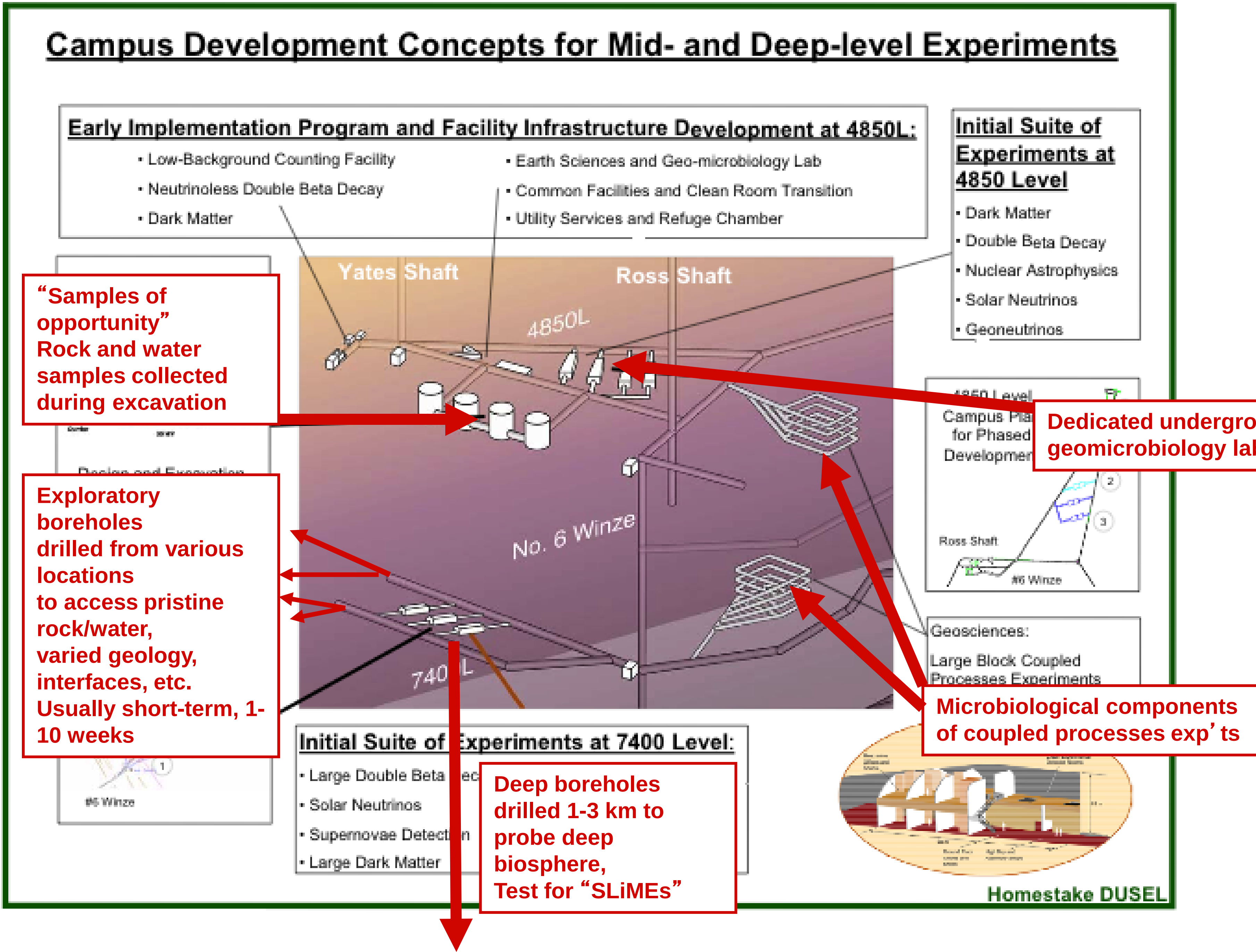
Subsystems Download chart data
has 12,446,285 predicted functions
169.2% of predicted proteins
304.1% of annotated proteins
View Subsystems interactive chart

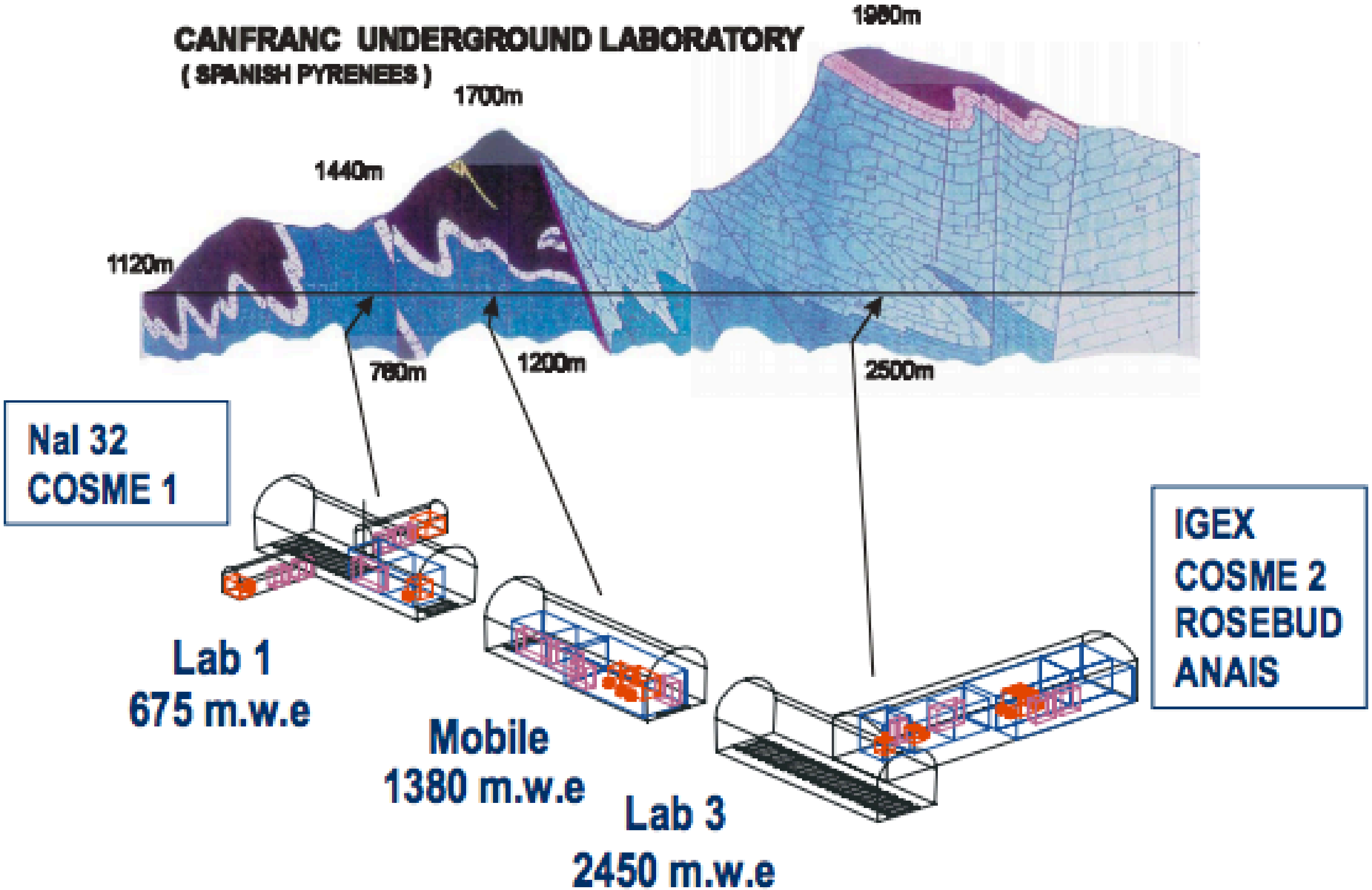


Maggie Lau, Princeton

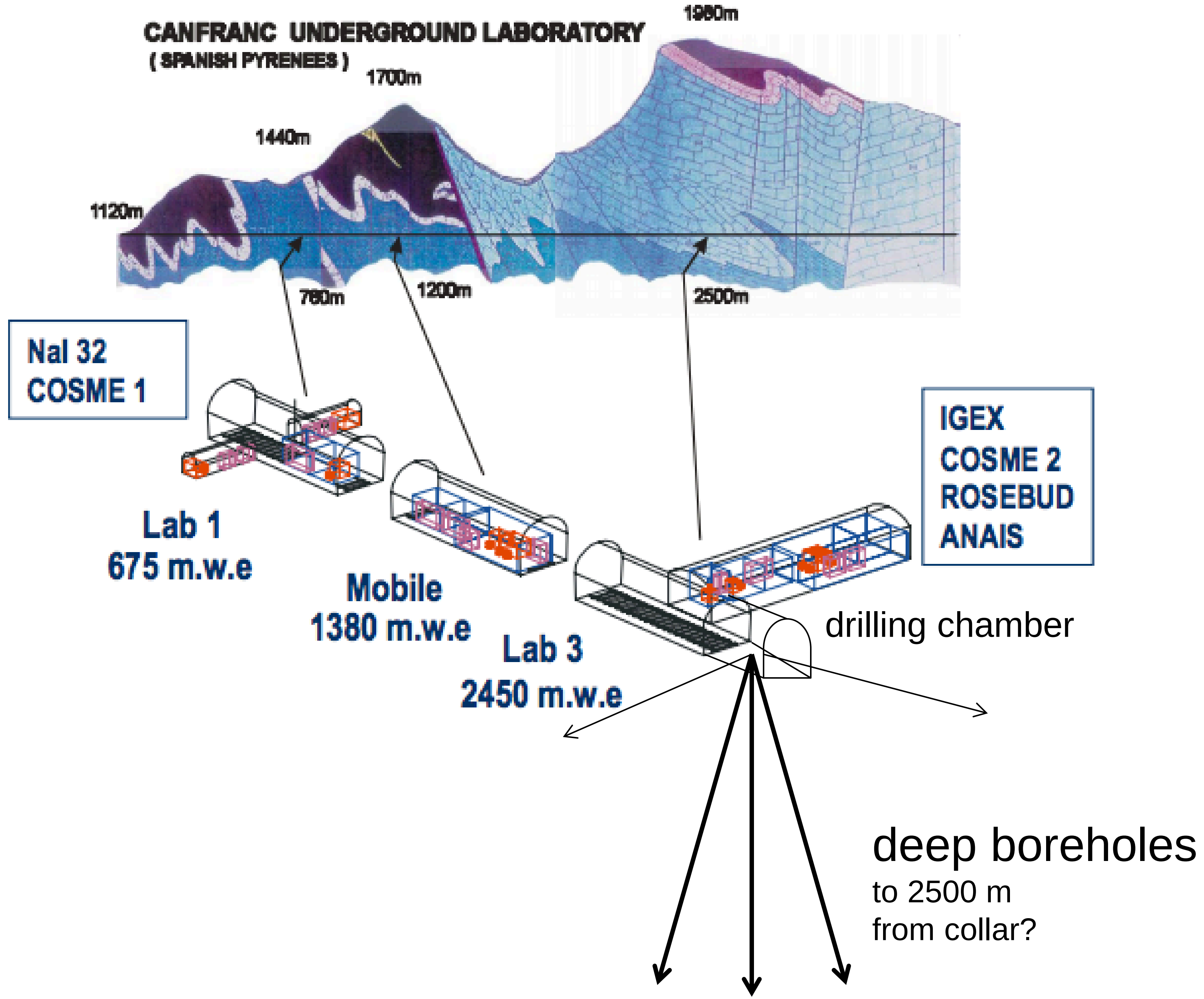

DUSEL
Deep Underground Science and Engineering Laboratory
at Homestake, SD
Six and a half Empire State Buildings for scale
Shallow Lab
Mid-level
Deep Campus
300 ft
4,850 ft
8,000 ft
Engineering
Geoscience
Biology
Physics
Astrophysics
N
P
NSF

Homestake DUSEL plans for Deep Life studies (suspended in 2010):



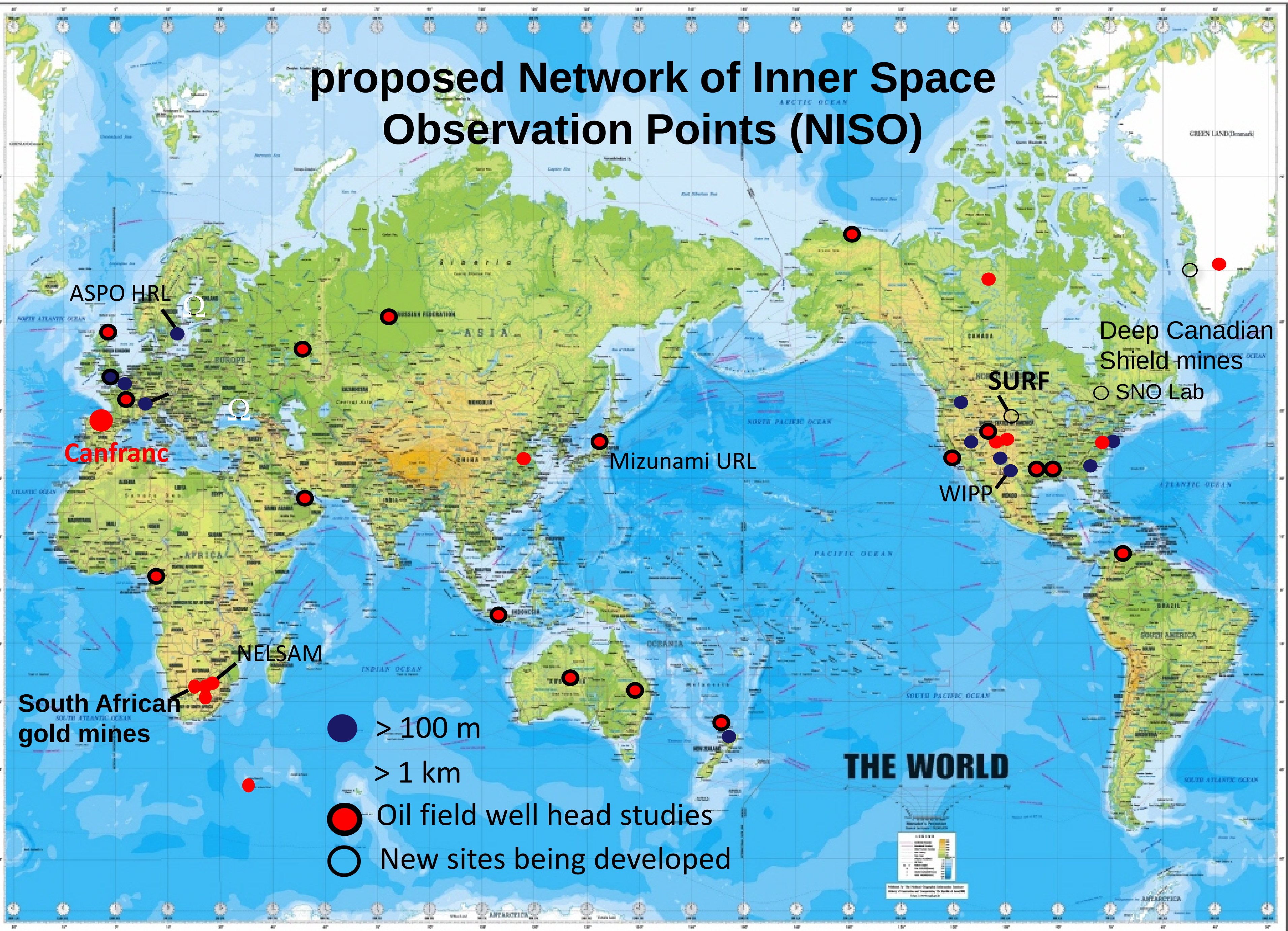


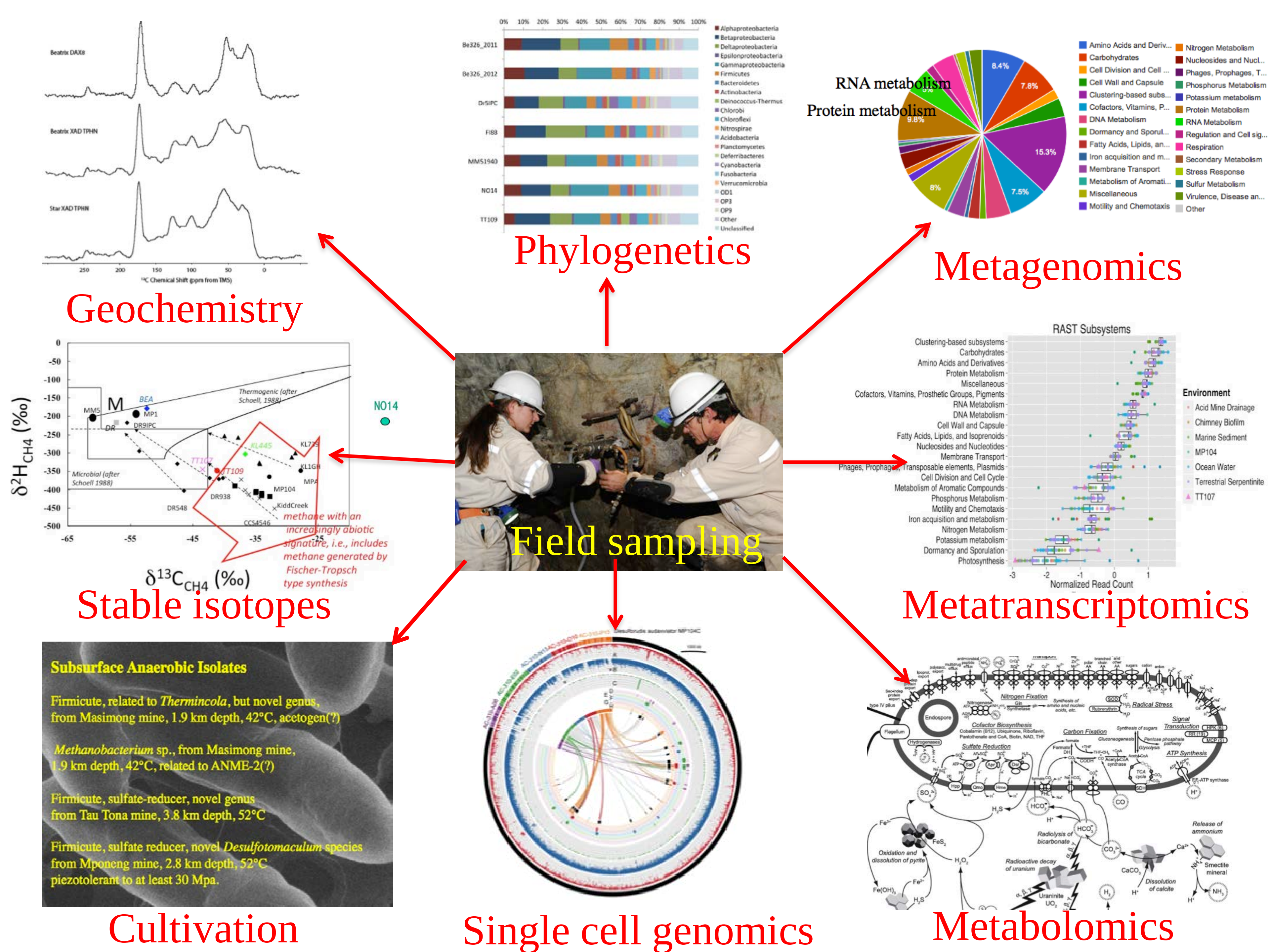
CANFRANC UNDERGROUND LABORATORY
(SPANISH PYRENEES)
1980m
1700m
1440m
1120m
780m
1200m
2500m
NaI 32
COSME 1
Lab 1
675 m.w.e
Mobile
1380 m.w.e
Lab 3
2450 m.w.e
IGEX
COSME 2
ROSEBUD
ANAIS



CANFRANC UNDERGROUND LABORATORY
(SPANISH PYRENEES)
1980m
1700m
1440m
1120m
760m
1200m
2500m
NaI 32
COSME 1
Lab 1
675 m.w.e
Mobile
1380 m.w.e
Lab 3
2450 m.w.e
IGEX
COSME 2
ROSEBUD
ANAIS
drilling chamber
deep boreholes
to 2500 m
from collar?

Mobile Underground Laboratory and Experimentation (MULE)


proposed Network of Inner Space Observation Points (NISO)
ASPO HRL
Ω
Ω
Canfranc
Mizunami URL
Deep Canadian Shield mines
SURF
SNO Lab
WIPP
NELSAM
South African gold mines
> 100 m
> 1 km
Oil field well head studies
New sites being developed
THE WORLD


Geochemistry
Phylogenetics
Metagenomics
RNA metabolism
Protein metabolism
Stable isotopes
Field sampling
RAST Subsystems
Metatranscriptomics
Subsurface Anaerobic Isolates
Firmicute, related to Thermincola, but novel genus, from Masimong mine, 1.9 km depth, 42°C, acetogen(?)
Methanobacterium sp., from Masimong mine, 1.9 km depth, 42°C, related to ANME-2(?)
Firmicute, sulfate-reducer, novel genus from Tau Tona mine, 3.8 km depth, 52°C
Firmicute, sulfate reducer, novel Desulfotomaculum species from Mponeng mine, 2.8 km depth, 52°C piezotolerant to at least 30 Mpa.
Cultivation
Single cell genomics
Metabolomics
methane with an increasingly abiotic signature, i.e., includes methane generated by Fischer-Tropsch type synthesis
Thermogenic (after Schoell, 1988)
Microbial (after Schoell 1988)
N014

Targets of interest

- Access to multiple locations with varied geology
- Access to locations with geological interfaces, geochemical gradients
- Access to pristine "green fields" (unmined, unimpacted by mining)
- Access at multiple depths
- Access to a deep site (2-3 km) from which to drill/core as deeply as possible
- Access to ancient groundwater (> 1 Ma, preferably >100 Ma)
- Potential for abiotic H_2